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Effective DNA epitope chimeric vaccines for Alzheimer's disease using a toxin-derived carrier protein as a molecular adjuvant

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Abstract Active amyloid-beta (Aβ) immunotherapy is under investigation to prevent or treat Alzheimer disease (AD). We describe here the immunological characterization and protective effect of DNA epitope chimeric vaccines using 6 copies of Aβ1-15 fused with PADRE or toxin-derived carriers. These naked 6Aβ15-T-Hc chimeric DNA vaccines were demonstrated to induce robust anti-Aβ antibodies that could recognize Aβ oligomers and inhibit Aβ oligomer-mediated neurotoxicity, result in the reduction of cerebral Aβ load and Aβ oligomers, and improve cognitive function in AD mice, but did not stimulate Aβ-specific T cell responses. Notably, toxin-derived carriers as molecular adjuvants were able to substantially promote immune responses, overcome Aβ-associated hypo-responsiveness, and elicit long-term Aβ-specific antibody response in 6Aβ15-T-Hc-immunized AD mice. These findings suggest that our 6Aβ15-T-Hc DNA chimeric vaccines can be used as a safe and effective strategy for AD immunotherapy, and toxin-derived carrier proteins are effective molecular adjuvants of DNA epitope vaccines for Alzheimer's disease.

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1. Introduction

The amyloid cascade hypothesis suggests that the production and accumulation of excessive amyloid-β (Aβ) may be the main cause in the onset and progression of Alzheimer's disease (AD) [1,2]. Current data also suggest that the accumulation of neurotoxic Aβ (soluble Aβ oligomers) may play a central role in the onset and progression of AD [2,3]. More and more researches reported that active Aβ vaccination can effectively clear the cerebral Aβ load in various AD mouse models and AD patients [4–8]. However, meningoencephalitis was observed in the first clinical AD vaccination trial (AN-1792), and only a small subset of immunized AD patients (19.7%) generated low/moderate titers of anti-Aβ antibodies, while approximately 50% of patients failed to produce measurable antibody responses [6,7]. Aβ-specific T cell-mediated autoimmunity produced by the C-terminal portion of Aβ42 might be the main cause leading to above adverse effects [9,10]. The low anti-Aβ antibody titers and non-responsiveness observed in the trial of AN-1792 could be due to immune tolerance induced by the Aβ42 self-antigen or hypo-responsiveness in elderly people [4,5,11]. As high titers of anti-Aβ antibodies would be therapeutically beneficial in AD patients [6,12], the development of a safe and effective AD vaccine requires a delicate balance between providing therapeutically adequate anti-Aβ or oligomeric Aβ antibody responses and eliminating unwanted adverse T cell-mediated autoimmune responses.

A promising and efficacious strategy to avoid these problems is active vaccination with an epitope vaccine composed of the immunodominant B cell self-epitope of Aβ42 [4,5,13]. Many peptide-based epitope vaccines, by attaching various N-terminal Aβ-specific B-cell epitopes to the promiscuous foreign T helper (Th) epitope pan HLA DR-binding peptide (PADRE), have been reported to be effective and safe in wild-type and APP/Tg mice [14–16]. DNA immunization provides an attractive alternative to direct peptide epitope and adjuvant approaches to induce an adequate anti-Aβ antibody response in the absence of potentially adverse self T cell-mediated events [11,17–19]. In the present report, we generated and tested the immunogenicity and protective efficacy of DNA Aβ1-15 epitope chimeric vaccines containing a toxin-derived carrier, which were designed to boost humoral responses and avoid the activation of Aβ-specific T-cells. Our results clearly demonstrated that prophylactic immunizations with the 6Aβ15-T-Hc DNA chimeric vaccines without gene gun or electroporation could induce high titers of anti-Aβ antibodies but not stimulate Aβ-specific T cell responses. More, these DNA epitope chimeric vaccines could reduce the amyloid accumulation and prevent the development of behavioral deficits in AD mice without unwanted side effects.

2. Materials and methods

2.1. DNA constructions

Initially, a chimeric 6Aβ15-T minigene containing 6 copies of the immunodominant B-cell epitope peptide Aβ1-15 (Aβ15) separated from each other by a GS small linker and PADRE was synthesized and cloned into the pMD18 DNA plasmid vector by Invitrogen Biotechnology Co., Ltd (Shanghai, China). Then, the minigene was cloned into plasmid pVAX1, resulting in recombinant plasmid pVAX1-6Aβ15-T (Supplementary Fig. S1). Further, a set of DNA plasmids including pVAX1-6Aβ15-T-AHc, pVAX1-6Aβ15-T-AHc-C, pVAX1-6Aβ15-T-THc or pVAX1-6Aβ15-T-THc-C (*i.e.*, 6Aβ15-T-Hc/Hc-C DNA chimeric vaccines) were constructed by fusing the genes of AHc or AHc-C of BoNT/A [20,21], THc or THc-C of TeNT [22] to C-terminus of 6Aβ15-T with G4SG4S small linker, respectively (Supplementary Fig. S1A).

All plasmids were prepared and purified using Endofree Mega-Q kits (QIAGEN GmbH, Hilden, Germany) for immunization. The expression of the plasmid *in vitro* was detected in transfected BHK-21 cells by indirect immunofluorescence assay (IFA). Briefly, BHK-21 cells at 70–80% confluency were transfected with complexes of plasmid DNAs and Lipofectamine® 2000 (Invitrogen, CA) following the manufacturer's instructions. Transfected cells were maintained in DMEM with 10% (v/v) heat-inactivated FCS (Invitrogen) for 24 h in a humidified 5% CO2 chamber. Cells were fixed with methanol, and antigen-positive cells were visualized by IFA using anti-β-amyloid monoclonal antibody (BAM-10, Sigma, St. Louis, MO) and fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse secondary antibody.

2.2. Immunization

Specific pathogen-free female BALB/c and C57/BL6 mice (purchased from Beijing Laboratory Animal Center, Beijing, China) at eight weeks of age were randomly assigned to different treatment groups. Groups of eight mice were i.m. immunized with 30 μg of plasmid DNA vaccines in a total volume of 0.1 ml and boosted 3 or 5 times by the same method biweekly. As negative controls, mice were injected with 30 μg of pVAX1 vector as above. Human Aβ1-42 (Aβ42) and Aβ1-15 (Aβ15) peptides (synthesized by Beijing Protein Innovation Co., Ltd., Beijing, China) were resuspended at a concentration of 10 mM in dimethyl sulfoxide (DMSO) and then diluted in PBS to obtain a 2 mg/ml peptide stock solution. Each peptide (10 μg/injection) formulated with 10% (v/v) aluminum hydroxide (Sigma) was used to vaccinate mice in parallel groups. Repeated studies were performed and similar results were obtained in one additional experiment.

PDAPP^{V717I} transgenic mice (derived from parental C57/BL6 mice) [23], used as AD model animals in the vaccination study, were obtained from the Institute of Experimental Animals, Chinese Academy of Medical Sciences & Peking Union Medical College (Beijing, China). Groups of 8 mice (3 months old, 28 ± 2 g) were vaccinated i.m. with 30 μg plasmid DNA vaccines (100 μl/injection) as above. Three vaccinations were performed at 2-weekly intervals, and three boosters were given at one or two month interval as indicated in Supplementary Fig. S1C. The animal protocols in this study were approved by our Institutional Animal Care and Use Committee.

2.3. Detection of antibody responses

Sera from mice 4 weeks after the final vaccination were screened for anti-Aβ42, Aβ15 or Aβ16-42 antibodies by ELISA. Briefly, ELISA plates (Corning Inc., Corning, NY) were coated